Karyotype studies made following the acetoorcein technique disclosed 33 somatic chromosomes which can be arranged into 11 triplets (figures 1, 2) of which 1, 2, 5, 7, 8, 9, 10 and 11 are heteromorphic and 3, 4 and 6 are homomorphic. In heteromorphic ones, it has been possible to group the chromosomes into pairs and singles indicating hybrid origin from tetraploid and diploid ancestors. In general there are 25 long and 8 medium chromosomes ranging from 5.59 to 15.18 μm with a total chromatin length of 329.38 μm. The karyotype is asymmetrical with 8 median, 6 submedian and 19 subterminal chromosomes. Triplets 1 and 7 possess secondary constrictions on their long arms.

A. belladonna was investigated earlier[1-9] and all the investigators reported $2n = 22$ chromosomes except Fernandes who observed the number $2n = 20$. The present report of $2n = 33$ chromosomes is a new number for the species. The karyotypic data suggest that this taxon is an allotriploid, the origin of which can be traced to a cross between a diploid and a tetraploid. Comparative morphological data on diploid and triploid *A. belladonna* plants reveal that triploidy results in bigger bulbs (7.9 cm, 12.5 cm), larger leaves (L/B 10.5/0.8 cm, 15.31/1.44 cm) and greater height (17.4 cm, 25.8 cm) but smaller stomata (L/B 38.25/12.57 μm, 30.61/10.71 μm).

24 May 1984

1. Fernandes, A., *Bol. Soc. Broteriana*, 1931, Ser. 2, 3.
2. Ficker, T., *Plant Life*, 1951, **7**, 68.
3. Inariyama, S., *Karyotype studies in Amaryllidaceae, Int. Sci. Rep. Tokyo Univ.*, 1937, Sec. **B3**, 95.
4. Kapoor, B. M. and Tandon, S. L., *Cytologia*, 1963, **28**, 399.
5. Larsen, K., *K. Danske, Videnskab. Biol. Skr.*, 1960, **11**, 1.
6. Sato, D., *Cytologia*, 1938, **9**, 203.
7. Sato, D., *Jpn J. Bot.*, 1942, **12**, 57.
8. Sharma, A. K. and Jash, M., *Phyton (Buenos Aires)*, 1958, **11**, 103.
9. Traub, H. P., *The Amaryllis manual*, Macmillan, New York, 1958, p. 338.

NITROGEN FIXATION (C_2H_2 REDUCTION) IN THE RICE RHIZOSPHERE SOIL AS INFLUENCED BY PESTICIDES AND FERTILIZER NITROGEN

J. L. N. RAO, J. S. PRASAD and
V. RAJARAMAMOHAN RAO
Central Rice Research Institute, Cuttack 753006, India.

USE of pesticides and fertilizer nitrogen has become an integral part of crop production. These agrochemicals exert profound influence on certain important biological transformations[1-4] of which nitrogen fixation is of topical interest[1, 2, 5]. Several reports indicate the stimulatory and inhibitory effects of commonly used pesticides on biological nitrogen fixation[1-4, 6, 7]. It has been established that high levels of mineral nitrogen inhibit the soil nitrogenase activity in paddy soils[8]. It is a common practice to apply mineral fertilizers and pesticides together in rice culture to achieve higher yields. We report the effects of three pesticides on the rice rhizosphere soil nitrogenase with and without mineral fertilizers.

A greenhouse study to evaluate the relative effects of three commonly used pesticides [carbofuran, (2,3 dihydro-2,2-dimethyl-7-benzofuranyl N-methyl carbamate); prophos, (0, ethyl SS-diprophyl phosphorodithioate) and metham sodium (sodium N-methyl dithiocarbamate)] having both insecticidal and nematicidal properties with and without nitrogen fertilizer on the rhizosphere soil nitrogenase was conducted during 1983. Carbofuran and prophos were applied at 2 kg a.i./ha and metham sodium at 500 l/ha as soil drench to the 5 kg soil in pots. Fertilizer nitrogen as urea at the rate of 60 kg N/ha equivalent as a basal dressing was applied at the time of transplanting. All treatments including control were replicated thrice. Rhizosphere soil (2 g fresh weight) was collected from three plants from each pot (3 pots for each treatment) and transferred to 125 × 16 mm B–D vacutainer tubes for C_2H_2 reduction analysis. The incubation and nitrogenase analysis were conducted as per the details described earlier[6, 7] on a gas chromatograph fitted with hydrogen flame ionization detector.

Nitrogenase activity varied throughout the growing period, indicating the influence of the plant growth phase. Ample evidences exist to show that rice plant influences the rhizosphere soil nitrogenase activity[5, 6]. Soil application of the three insecticides stimulated the nitrogenase activity. Prophos effected the highest stimulation almost throughout the growing season (table 1).

Table 1 *Influence of pesticides and fertilizer on the rice rhizosphere soil nitrogenase activity*

Treatment		n moles of C_2H_4 formed g^{-1} soil day^{-1} Days after transplanting					
		30	40	48	60	75	83
Unplanted without fertilizer	Control	406	154	254	143	40	58
	Carbofuran	783	489	308	243	50	69
	Metham sodium	588	182	285	272	92	47
	Prophos	1347	596	221	270	147	142
	L.S.D. 5%	190	78	NS	49	27	25
	1%	261	107		67	37	34
Planted without fertilizer	Control	454	176	158	165	54	56
	Carbofuran	414	355	453	210	106	75
	Metham sodium	511	550	440	137	122	81
	Prophos	743	683	300	468	61	121
	L.S.D. 5%	165	153	108	92	30	NS
	1%	228	149	149	126	41	
Planted* with fertilizer	Control	593	223	360	176	24	43
	Carbofuran	900	263	227	279	69	53
	Metham sodium	976	263	238	407	88	167
	Prophos	1202	360	430	286	86	57
	L.S.D. 5%	323	94	104	111	31	31
	1%	445	NS	144	153	43	43

* Nitrogen at 60 kg N ha as urea was applied. Carbofuran and prophos were applied at 2 kg a.i./ha and metham sodium at 500 i ha as soil drench.

The effect of pesticides remained identical in planted system especially with regard to stimulation by prophos. Nitrogenase was stimulated in the presence of fertilizer and pesticides at least in the initial (30 days) samplings. However, application of higher levels (60 kg N/ha) of nitrogen along with pesticides resulted in the inhibition of soil nitrogenase in several subsequent samplings. Thus the rhizosphere nitrogenase was low when assessed 40 days after transplanting and throughout the remaining growing season when fertilizer nitrogen was applied. In fact, fertilizer nitrogen when applied in levels beyond 40 kg N/ha inhibited nitrogen fixation in several paddy soils[8]. Interestingly, the stimulation by pesticides in presence of fertilizer nitrogen was highest only during the early sampling; but in subsequent samplings the activity remained low as compared to the unfertilized planted system. The nitrogen fixation rates reported are, however, suggestive owing to long term incubation.

Significant stimulation of soil nitrogenase due to pesticide application has been demonstrated in rice culture[1, 2, 6, 7]. The stimulation was more uniform with carbofuran in several studies[1]. In the present study application of carbofuran effected significant stimulation in many samplings. In addition, prophos, essentially a non-systemic nematicide, exerted considerable stimulation of rhizosphere soil nitrogenase. Another nematicide metham sodium also stimulated soil nitrogenase but to a lesser extent compared to prophos. These studies indicate that certain soil applied nematicides stimulate soil nitrogenase besides being nematicidal.

The authors thank Dr H. K. Pande, Director, for his keen interest and encouragement. Financial assistance from UGC is acknowledged.

28 March 1984; Revised 14 June 1984

1. Nayak, D. N. and Rao, V. R., *Soil Biol. Biochem.*, 1982, **14**, 207.
2. Raghu, K. and MacRae, I. C., *Can. J. Microbiol.*, 1967, **13**, 622.
3. Simon-Sylvestre, G. and Fournier, J. C., *Adv. Agron.* 1979, **31**, 1.
4. Tu, C. M. and Miles, J. R. W., *Residue Rev.*, 1976, **14**, 17.
5. App, A. A., Watanabe, I., Alexander, M., Ventura, W., Daez, C., Santiago, I. and De Datta, S. K., *Soil Sci.*, 1980, **130**, 285.

6. Mohapatra, R. N. and Rao, V. R., *Plant Soil.*, 1981, **59,** 473.
7. Nayak, D. N., Pasalu, I. C. and Rao, V. R., *Curr. Sci.*, 1980, **49,** 118.
8. Charyulu, P. B. B. N. and Rao, V. R., *Soil Sci.*, 1979, **128,** 86.

CHANGES IN ISOENZYMES OF PEROXIDASE IN GREEN-EAR OF PEARL MILLET

N. S. SHEKHAWAT, S. D. PUROHIT and H. C. ARYA
Department of Botany, University of Jodhpur, Jodhpur 342001, India.

ALTERED state of oxidative enzymes plays important role in plant metabolism during pathogenesis[1]. *Sclerospora graminicola* causes proliferations in ear-heads of *Pennisetum americanum* (called green-ear of pearl-millet) and affects the activities of oxidative enzymes[2,3]. We have reported higher peroxidase and lower IAA-oxidase activities parallel with increased protein, total phenols, orthodihydroxyphenols, and auxin contents in the diseased millet tissues[2]. We report the isoenzymatic patte.n of peroxidase in healthy and diseased millet tissues.

Peroxidase isoenzymes were separated by zone electrophoresis in polyacrylamide gel using method of Davis[4] and Ornstein[5]. Acetone powder of various sample categories was homogenized in 0.05 Tris-HCl buffer (pH 7.8). Supernatant fraction after centrifugation at 15,000 rpm was taken as representative sample containing 50–60 μg of protein and mixed with 60% sucrose (1:2) to omit use of sample and spacer gels. It was then layered on the top of running gel (7.5%). Cathode and anode were connected to upper and lower reservoirs respectively. A constant current of 3 m A/tube (150 Volts) was applied, the electrophoresis was accomplished in 70–75 min.

Gels were stained with saturated solution of benzidine (in 25% acetic acid) and 1% H_2O_2 for peroxidase activity. The dark blue bands appearing within 1–2 min were noted.

Isoenzyme pattern was studied at various stages of the disease. In the healthy leaves five anodic isoenzymes (gel-1; A_2, A_3, A_{10}, A_{12} and A_{13}) were recorded which increased to eight with three new

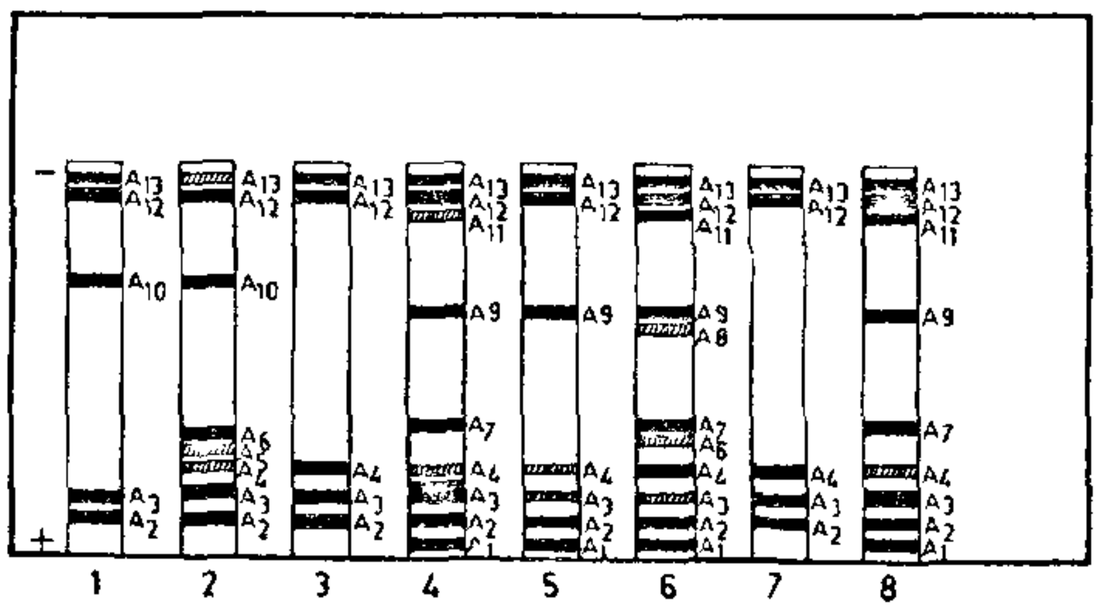

Figure 1. Peroxidase isoenzyme pattern in different stages of green-ear in pearl-millet. Gel-1. Healthy leaf, **2.** Diseased leaf, **3.** Healthy ear-head, **4.** Green-ear initial stage, **5.** Completely proliferated ear-head, **6.** Suppressed ear-head, **7.** Healthy half of half-deformed ear-head, **8.** Diseased half-deformed ear-head. ▬ Deep-stained bands, ▨ Light stained bands.

bands (gel-2; A_2, A_3, A_4, A_5, A_6, A_{10}, A_{12} and A_{13}) in diseased leaves.

In healthy ear-head and the healthy half of the half-deformed ear-head five isoenzyme bands (gel-3, 7; A_2, A_3, A_4, A_{12} and A_{13}) were noted. In the green-ear initial stage and diseased half of half-defomred ear-head nine isoenzymes were recorded (gel-4, 8; A_1 to A_4, A_7, A_9 and A_{11}–A_{13}) out of which three (A_1, A_7 and A_9) were new as compared to healthy ear-head (gel-3) and healthy half of half-deformed ear-head (gel-7). In completely proliferated ear-head, seven (gel-5; A_1 to A_4, A_9, A_{12} and A_{13}) isoenzymes band were noted. The isoenzyme A_7, common in the other three stages of diseased ear-heads, disappeared in this stage. The suppressed ear-heads showed eleven isoperoxidase (gel-6; A_1 to A_4, A_6, A_7, A_8, A_9 and A_{11} to A_{13}).

We have reported highest peroxidase activity in the suppressed ear-heads followed by green-ear initial stage, diseased half of half-deformed ear-heads, completely proliferated ear-heads and diseased leaves over their healthy counter parts[2]. The number of isoenzymes followed the same line. Stahmann and Demorest[6] concluded that appearance of new isoperoxidases was because of *de novo* protein synthesis in the parasitized tissues. Presumably, the appearance of new isoperoxidases and the higher peroxidase activity in the diseased tissues was also a result of higher protein levels recorded in the affected tissues[3].

Shekhawat and Arya[2] reported suppressed IAA-oxidase activity in the diseased tissues (lowest being in